Attractant and Disruptant Semiochemicals for *Dendroctonus jeffreyi* (Coleoptera: Curculionidae: Scolytinae)

B. L. STROM,1,2 S. L. SMITH,3 AND C. BROWNIE4

ABSTRACT Jeffrey pine, *Pinus jeffreyi* Greville and Balfour, is a dominant yellow pine and important overstory component of forests growing on diverse sites from southwestern Oregon to Baja California to western Nevada. The Jeffrey pine beetle, *Dendroctonus jeffreyi* Hopkins (Coleoptera: Curculionidae: Scolytinae), is monophagous on Jeffrey pine and its primary insect pest. Despite the importance of *P. jeffreyi*, difficult terrain, environmental concerns, and lack of roads can constrain pest management activities. Semiochemicals are often easier to apply and more environmentally acceptable than other options, but they are lacking in this system. Attractants have been identified, but field bioassays have been limited because of infrequent or short duration outbreaks and a lack of beetles during nonoutbreak periods. Disruptant semiochemicals have not been assessed for *D. jeffreyi* during outbreak conditions; however, commercially available semiochemicals have been implicated as disruptants for this bark beetle. The objective of this study was to identify the most effective commercially available attractant and disruptant semiochemicals for *D. jeffreyi*. Our highest observed catch occurred with the blend of 5% 1-heptanol and 95% n-heptane. When this was used to challenge potential disruptant semiochemicals, the combination of S-(−)-verbenone and the green leaf volatile blend (cis-3-Hexenol and 1-Hexanol) reduced trap catch by \( \approx 80\% \). However, frontalin was most effective, reducing the number of *D. jeffreyi* caught by \( \approx 96\% \). Within each year of the study, the percentage female of *D. jeffreyi* caught with our attractant decreased from start to end of the experimental period. On average, our first collection in a year (mid-June to early July) was 59% female, whereas our last (mid-August) was 34%. Frontalin was equally or more effective against females (the pioneering sex) than males, providing optimism that semiochemical disruption may be possible for protecting Jeffrey pines from *D. jeffreyi*.

KEY WORDS *Pinus jeffreyi*, tree protection, frontalin, pheromone, green leaf volatiles

Jeffrey pine, *Pinus jeffreyi* Greville and Balfour, is an important overstory component of forests in California and throughout its natural range, which extends from southwestern Oregon to northern Baja California and east to western Nevada (Jenkinson 1990). It competes well on cold, harsh sites (Jenkinson 1990) and large, high-value specimens enhance some of the most popular recreational areas in northern California. The Jeffrey pine beetle, *Dendroctonus jeffreyi* Hopkins 1909 (Coleoptera: Curculionidae: Scolytinae), is monophagous on Jeffrey pine and its primary insect pest (Smith et al. 2009). Populations of *D. jeffreyi* exhibit extreme “boom-or-bust” cycles, causing high levels of host mortality during extended droughts (Smith et al. 2009), but being limited in numbers at other times, as indicated by catches in attractant-baited traps or lack of host mortality (Renwick and Pitman 1979, Paine et al. 1999). During the late 2000s, increased host mortality caused by *D. jeffreyi* in the Lake Tahoe region provided an abundant population of beetles for practical measurement of semiochemical effects.

Numerous semiochemicals have been applied in forested ecosystems to mediate the host selection process of insects (Borden 1993). Insect host selection has been described as a catenary process with five steps: host-habitat finding, host finding, host recognition, host acceptance, and host suitability (Kogan 1994). It culminates in reproduction, after acceptance or rejection of a resource by each insect (Kennedy 1965, Miller and Strickler 1984). Semiochemicals may mediate the process; for example, attractants may enhance or focus host finding and disruptants may prevent its completion. Disruption may occur at any step, and via any one or more sensory modalities (gustation, hearing, olfaction, vision); this is an implicit goal of pest management. Other terms have been used to describe this primarily olfactory phenomenon with bark beetles (antiattractant, antiaggregant, inhibitor); we use disruptant because it remains descriptive across sensory modalities, uses a host selection framework, and consistently refers to individuals. Semio-
chemicals deployed as disruptants for bark beetles usually target host finding (or trap finding, a surrogate). Commercial products with activity against multiple species of *Dendroctonus* are available, but they have not been measured with *D. jeffreyi*.

Similar to other *Dendroctonus*, *D. jeffreyi* uses semiochemicals to promote mass attack of its host, a necessity for overwhelming host tree defenses, colonization, and beetle reproduction. Potential attractant semiochemicals for *D. jeffreyi* have been identified, but field bioassays have been limited by infrequent or short duration outbreaks and a paucity of beetles during nonoutbreak years. Based on concentrations present in hindgut tissues, Pitman et al. (1969) list brevicom in as a major component in male *D. jeffreyi* and trans-verbenol as a major component in females. They list minor components as trans-verbenol in males and cis-verbenol and brevicon in females. Paine et al. (1999) more recently identified the brevicomin in male *D. jeffreyi* as (+)-exo-brevicomin, but they did not detect it in females. Verbenone is produced by male *D. jeffreyi* (Pitman et al. 1969) and is also present in host volatiles (Shepherd et al. 2008). Attacking female beetles (the pioneering sex in *Dendroctonus*) have the pheromones 1-heptanol and 2-heptanol as major components (Renwick and Pitman 1979). These pheromones are oxidized products of n-heptane, which accounts for ≈95% of the volatile component of host oleoresin (Smith 2000). Renwick and Pitman (1979) found that 1-heptanol, in combination with n-heptane, was attractive to *D. jeffreyi*, whereas 2-heptanol was not. However, this observation was based on only 125 beetles trapped during their 6-wk study. Paine et al. (1999) confirmed the attraction of 1-heptanol and n-heptane to *D. jeffreyi* at apparently low beetle populations, and also found that female catch was enhanced by the addition of racemic *exo*-brevicomin.

With the possible exception of frontalin (Paine et al. 1999), disruptant semiochemicals have not been specifically identified for *D. jeffreyi*. Frontalin is common in *Dendroctonus*, is produced by male *D. jeffreyi* (Paine et al. 1999, Hall et al. 2002), and was found to reduce trap catch of females (but not males) when attractant-baited traps caught relatively low numbers (2.8 beetles per day; Paine et al. 1999). Verbenone disrupts multiple *Dendroctonus* species, including *D. valens* LeConte (Gillette et al. 2001) and *D. ponderosae* Hopkins (Ryker and Yandell 1983), a species closely related to *D. jeffreyi* (Hopkins 1909). The so-called nonhost or green leaf volatiles (GLV herein; see Zhang and Schlyter 2004 for review) have broadband spectrum activity with scolytids, often synergizing the effects of verbenone or allowing reduced dosages to be deployed (e.g., Wilson et al. 1996, Borden et al. 1998, Zhang and Schlyter 2004). Verbenone holds a United States Environmental Protection Agency registration for use against some species of bark beetles and GLV blends are available from commercial vendors for research purposes.

Consistent efficacy is a major concern with deploying disruptant semiochemicals against bark beetles, but the localities in which Jeffrey pines occur make them an attractive option for protecting high-value resources threatened by *D. jeffreyi*. Spraying of tree boles with synthetic insecticides is generally effective against *Dendroctonus* bark beetles in western North America (Haverty et al. 1998, Fettig et al. 2006) and carbaryl, in particular, has shown toxic activity to *D. jeffreyi* in log assays (Smith 1982). In addition, circumstantial evidence suggests that bole sprays are effective for protecting host trees against *D. jeffreyi* (Smith et al. 2009), but difficulties remain for areas in which this is not a viable treatment option. Prevention of *D. jeffreyi* impacts through thinning can be effective at the stand level (Egan et al. 2011); however, a more targeted, specific tree method is sought for high-value trees when acutely threatened. In addition, significant limitations exist to the application of either bole sprays or thinning treatments to *P. jeffreyi* (e.g., terrain, environmental concerns), making the identification of an effective disruptant semiochemical a pest management priority for this system.

The objective of this study was to assess semiochemicals for manipulating *D. jeffreyi* in areas with significant, current beetle-caused mortality of Jeffrey pine. These environmental conditions were available during the late 2000s with increased activity of *D. jeffreyi* in the Lake Tahoe region. We performed four sequential funnel trap bioassay experiments to identify the best attractant for *D. jeffreyi* and then used it to measure commercially available disruptants, including S-(−)-verbenone, GLV (a blend of cis-3-Hexenol and 1-Hexanol), and frontalin. Nontarget effects on insect behavior are of interest when semiochemicals are deployed, so common insect predators and other *Dendroctonus* species also were tallied from trap collections.

**Materials and Methods**

Experiments were conducted in California, near Lake Tahoe on the Lake Tahoe Basin Management Unit and the Humboldt-Toiyabe National Forest (both USDA Forest Service) at Luther Pass (elevation ≈2,358 m). Forests in the area were dominated by mixed conifer and second-growth Jeffrey pine stands, consisting of large trees (>50 cm diameter breast height) growing singly or in small clusters. From 2006 to 2008, droughty conditions in the area (monthly Palmer Drought Index ranged from −0.28 in May of 2006 to −3.48 in February of 2009; http://www1.ncdc.noaa.gov/pub/data/cirs/drd964x.pdsi.txt) coincided with increased tree mortality caused by *D. jeffreyi* from 2006 to 2009 (4.2 trees per hectare, Aerial Detection Monitoring Program, USDA Forest Service, Region 5, Forest Health Protection, Vallejo, CA; http://www.fs.usda.gov/detail/r5/forest-grasslandhealth/?cid=fbsd3 046696) and provided suitable conditions for measuring semiochemicals for their potential in managing *D. jeffreyi*.

A series of four experiments was conducted from 2007 to 2009 to measure attractant and disruptant effects of semiochemicals for *D. jeffreyi*; each exper-
Table 1. Estimated average release rates of semiochemicals from devices used in this study

<table>
<thead>
<tr>
<th>Device</th>
<th>Chemical</th>
<th>Test Location</th>
<th>Dates</th>
<th>Test Duration</th>
<th>Temperaturea</th>
<th>Mass Loss/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>250-ml bottle (Lure A)</td>
<td>95:5 heptane: heptanol</td>
<td>Luther Pass, CA</td>
<td>19 July-16 Aug. 2007</td>
<td>29 d</td>
<td>18.7d</td>
<td>~1 g</td>
</tr>
<tr>
<td>Synergy 16-ml bottle</td>
<td>Heptane</td>
<td>Pineville, LA</td>
<td>17 April–4 May 2007</td>
<td>~2 wk</td>
<td>20.2</td>
<td>~550 mg</td>
</tr>
<tr>
<td>APTf</td>
<td>95:5 heptane: heptanol</td>
<td>Pineville, LA</td>
<td>31 Aug.–7 Sept. 2007</td>
<td>~1 wk</td>
<td>26.2</td>
<td>1.01 mgc</td>
</tr>
<tr>
<td>Synergy high heptanol</td>
<td>Heptanol</td>
<td>Pineville, LA</td>
<td>31 Aug.–25 Oct. 2007</td>
<td>~2 mo</td>
<td>23.7</td>
<td>~60 mgc</td>
</tr>
<tr>
<td>Synergy low heptanol</td>
<td>Heptanol</td>
<td>Pineville, LA</td>
<td>31 Aug.–25 Oct. 2007</td>
<td>~2 mo</td>
<td>23.7</td>
<td>~10 mgc</td>
</tr>
<tr>
<td>Synergy exo-brevicomin</td>
<td>exo-brevicomin</td>
<td>--</td>
<td>--</td>
<td>20</td>
<td>125 pgc</td>
<td></td>
</tr>
<tr>
<td>Synergy pouch</td>
<td>S-(-)-verbenone</td>
<td>Missoula, MT</td>
<td>5 June–21 Sept. 2007</td>
<td>50 d</td>
<td>20.1</td>
<td>66 mgc</td>
</tr>
<tr>
<td>Synergy pouch</td>
<td>GLV</td>
<td>Missoula, MT</td>
<td>5 June–21 Sept. 2007</td>
<td>50 d</td>
<td>20.1</td>
<td>97 mgc</td>
</tr>
<tr>
<td>Synergy microcentrifuge tubes</td>
<td>Frontalin</td>
<td>Pineville, LA</td>
<td>6 April–7 June 2007</td>
<td>62 d</td>
<td>17.6</td>
<td>6.5 mgc</td>
</tr>
</tbody>
</table>

Data were obtained during their use in these experiments, from other field-generated assessments, or from device suppliers.

a Average in degrees Celsius.


c Nalgene HDPE bottle, Thermo Fisher Scientific, Rochester, NY.

d From weatherunderground.com for listed dates; Tahoe Vista, CA, elevation 1,935 m. Accessed 13 Nov. 2007.

e Synergy Semiochemicals, Corp., Burnaby, BC, Canada.

f APTIV, Inc., Portland, OR.

g Full sun rate (http://www.fs.fed.us/foresthealth/technology/elutionrate/lure.htm).

h MPB ßex lure, rate supplied by Synergy Semiochemicals, Inc.

i 5-S(-)-verbenone pouch with 7.5-g load.

j GLV pouch with 10 g of the green leaf volatile blend cis-3-Hexenol and 1-Hexanol (Synergy Semiochemicals, Corp., personal communication).

k SPB lure, 600-mg load as two tubes with 300 mg each of racemic frontalin.

Experiment 1 was conducted from 15 June to 18 July 2007 and measured five semiochemical attractant treatments: blended heptane and heptanol (95:5 vol:vol) (APT; APTIV, Inc., Portland, OR); high release heptanol with heptane bottle (HRHH); high release heptanol, heptane bottle, and exo-brevicomin (HRHHE); low release heptanol and heptane bottle (LRRH); and low release heptanol, heptane bottle, and exo-brevicomin (LRHHE) (all from Synergy Semiochemicals, Corp.; Table 1). The blended APT lure required weekly replacement and heptane bottles (16 ml; Synergy Semiochemicals, Corp.) were refilled as needed with n-heptane (99%+, Acros Organics, Geel, Belgium). Twenty-five traps were deployed, providing five replicates per treatment. The experimental design also allowed for comparisons between treatments with high versus low heptanol and presence or absence of exo-brevicomin.

Experiment 2 was conducted later in 2007 (19 July to 16 August) and measured the disruptive effect of 5-S(-)-verbenone (BeekeBlock 7.5 g, Synergy Semiochemicals, Corp.) + GLV (a 10-g blend of cis-3-Hexenol and 1-Hexanol, Synergy Semiochemicals, Corp.) on D. jeffreyi. Both components disrupt host finding of a number of scolytid species, including D. ponderosae, although typically the GLV blend is more effective as an additive to verbenone than alone (Borden et al. 1998). Because there is no information on semiochemical disruption of D. jeffreyi (beyond the possibility of frontalin), we used components of the best available generic disruptive combination for pine-infesting Dendroctonus. The attractant lure in this experiment (Lure A) was an improved version of the APT lure from experiment 1, and was the same for all traps. It consisted of a 250-ml brown bottle (Nalgene HDPE bottle, Thermo Fisher, Inc., Rochester, NY), loaded with 100 ml of blended (95:5 vol:vol) n-heptane (99%+, Acros Organics): heptanol (99%, Alfa-
Aesar, Ward Hill, MA). A single hole (0.36 cm) was drilled into the cap for semiochemical emission. This provided a release rate similar to that of the APT lure in experiment 1 (Table 1), but one that did not require weekly replacement during the experiment. Twenty-four traps were deployed, providing 12 replicates per treatment.

The final two experiments were conducted in 2008 (experiment 3) and 2009 (experiment 4), and included racemic frontal (Synergy Semiochemicals, Corp.), a common *Dendroctonus* pheromone and one that was indicated previously as a disruptant for *D. jeffreyi* (Paine et al. 1999). In 2008, we deployed traps from 24 June to 5 August. Semiochemical treatments were: Lure A, Lure B (75:25 n-heptane: l-heptanol blend), Lure A + verbenone (two pouches), Lure A + verbenone + GLV (one pouch each), and Lure A + frontal (600-mg southern pine beetle load [2 microcentrifuge tubes at 300 mg each]). Each treatment was replicated five times for a total of 25 traps.

Experiment 4 was conducted from 29 June to 17 August 2009. This experiment focused on the effects of frontal and comparing the best disruptants from previous treatments. Lure A was included in every trap and was the lone treatment in six traps. Three additional treatments included one or more disruptant semiochemicals: 300-mg frontal (one microcentrifuge tube), 600-mg frontal, or 600 mg frontal with verbenone + GLV (one pouch each). Each of the four treatments was replicated six times for a total of 24 traps.

Experiments were designed primarily for analysis of total catch (i.e., trap sums) by treatment (one-way analysis of variance [ANOVA]). However, because we collected traps approximately weekly and identified the sex of each intact beetle, we began the data analysis for each experiment with a repeated measures (split-plot) ANOVA, using sex as the subplot factor, to estimate the importance of interactions between sex and treatments. When this interaction was not significant (NS, *P* > 0.05; all cases except experiment 1), we proceeded to use one-way ANOVA to determine treatment effects on total catch (trap sums) or percentage female. For experiment 1, we subjected the counts of *D. jeffreyi* to one-way ANOVA separately for each sex. Response variables were transformed as necessary by their square root, natural logarithm, or arcsine (whichever was considered best from examination of distributions and residual plots) before ANOVA, whenever doing so improved their ability to meet assumptions of normality and homogeneity of variances. Treatment means were subjected to Tukey’s honestly significant difference (HSD) to determine pairwise differences. To examine the relationship between collection period and sex ratio, the proportion female of *D. jeffreyi* caught was determined for each trap at each collection period for the attractant treatments. Random coefficient regression analysis (with trap and trap*date linear viewed as random) was carried out separately for the lures in each experiment to test for linear effects of collection period on the proportion of *D. jeffreyi* caught that was female.

The first collection of experiment 1 was a partial day collection, from the day of trap deployment, with one missing datum. To obtain trap sums, this single missing datum was imputed by fitting a full model with treatment, sex, collection date, and trap as model factors. Single degrees of freedom contrasts were used when the experimental design allowed measurement of a semiochemical used in combination or at different rates. In experiment 1, this included the presence or absence of exo-brevicomin and the high or low release rate of l-heptanol. In experiment 4, contrasts were used to measure the disruptive effects on sex ratio of *D. jeffreyi* and the effect of frontal on *D. valens* catch. All statistical analyses were conducted using SAS (version 9.2, SAS Institute, Cary, NC) or JMP (version 9.0.3, SAS Institute).

### Results

In experiment 1 (2007), a total of 3,349 *D. jeffreyi* (1,397 females; 1,924 males; 28 indeterminate), 278 *Temnochila chlorodia*, 113 *D. valens*, and 43 Cleridae were captured (Table 2). Because the split-plot ANOVA revealed that sex*treatment* was significant (*F*<sub>4,20</sub> = 5.21; *P* = 0.0048) for *D. jeffreyi*, treatment effects were analyzed separately by sex (Fig. 1). For females, semiochemical treatment significantly affected catch with the APT lure *X* = 117.6 ± 33.2; (mean ± 1 SEM, untransformed data) being highest, followed by HRHH (58.4 ± 15.4), HRHHE (46.4 ± 1.9), LRHH (38.2 ± 12.9), and LRHHE (18.5 ± 3.7). The only significant pairwise difference between treatment means was APT compared with LRHHE. However, lures that emitted high levels of heptanol (Table 1, APT, HRHH, HRHHE; *n* = 15) caught significantly more female *D. jeffreyi* than did low heptanol lures (LRHH and LRHHE, *n* = 10; *X* = 74.1 ±

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**Table 2.** Experiment 1. Mean catch of *D. jeffreyi* and associated insects by semiochemical treatment at Luther Pass, CA from 15 June to 18 July 2007 (*X* ± 1 SEM). Means within a column followed by different letters are significantly different by Tukey’s HSD (*P* < 0.05). Columns without letters contain no significantly different means

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. or % caught (mean per trap ± 1 SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>D. jeffreyi</em>&lt;sup&gt;b&lt;/sup&gt; % female&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>APT</td>
<td>252.8 ± 50.3</td>
</tr>
<tr>
<td>HRHH</td>
<td>163.6 ± 19.6</td>
</tr>
<tr>
<td>HRHHE</td>
<td>154.4 ± 11.5</td>
</tr>
<tr>
<td>LRHH</td>
<td>67.4 ± 21.4</td>
</tr>
<tr>
<td>LRHHE</td>
<td>36.1 ± 4.8</td>
</tr>
</tbody>
</table>

<sup>a</sup> APT = 95.5 n-heptanedi-heptanol (APIV, Inc., Portland, OR; HRHH = high release heptanol, heptane bottle; HRHHE = high release heptanal, heptane bottle, exo-brevicomin; LRHH = low release heptanol, heptane bottle; LRHHE = low release heptanal, heptane bottle, exo-brevicomin (all Synergy Semiochemicals, Corp., Burnaby, BC, Canada). See Table 1 for release rates.

<sup>b</sup> Sex<sup>+</sup> treatment was significant in 2007 to treatment effects were not evaluated for total number or percentage female of total number caught.
14.0 versus 28.5 ± 7.1; \( P = 0.006 \). There was no significant effect observed for exo-brevicomin on female \( D. \text{jeffreyi} \) (exo present, \( n = 10 \), \( \bar{X} = 32.6 \pm 5.0 \) versus exo absent, \( n = 15 \), \( \bar{X} = 71.4 \pm 14.0 \), \( P = 0.06 \)).

Males in experiment 1 also were affected by semiochemical treatment (Fig. 1). The APT lure caught the greatest number of male \( D. \text{jeffreyi} \) (\( \bar{X} = 134.4 \pm 18.9 \)), followed by HRHH (104.8 ± 12.2), HRHHE (103.8 ± 8.6), LRHH (29.0 ± 8.8), and LRHHE (12.8 ± 1.7). Significant differences were found between each high heptanol treatment (APT, HRHH, and HRHHE) compared with each low heptanol treatment (LRHHE and LRHHHE), but no differences were found within either group. As with females, the contrast comparing high to low heptanol treatments was significant for males (\( \bar{X} = 114.3 \pm 8.3 \) versus 20.9 ± 5.0, \( P < 0.0001 \)). Unlike females however, males were significantly affected by exo-brevicomin with fewer males being caught in traps with exo-brevicomin compared to traps without exo-brevicomin (exo present, \( n = 10 \), \( \bar{X} = 58.3 \pm 13.7 \) versus exo absent, \( n = 15 \), \( \bar{X} = 99.4 \pm 14.0 \), \( P = 0.012 \)).

Semiochemical treatment significantly affected the number of \( T. \text{chlorodia} \) caught in experiment 1 (\( F_{4,29} = 5.6, P = 0.003 \); Table 2). Treatment HRHHE caught by far the most \( T. \text{chlorodia} \), followed by LRHHE, APT, LRHH, and HRHH (Table 2). As expected (Bedard et al. 1969), the presence of exo-brevicomin (with exo, \( n = 10 \), \( \bar{X} = 23.2 \pm 9.5 \)) significantly increased catch of \( T. \text{chlorodia} \) compared with treatments without it (without exo, \( n = 15 \), \( \bar{X} = 3.1 \pm 1.1 \); \( df = 20 \), \( t = 3.66 \), \( P = 0.002 \)). Treatment means, and an interaction plot depicting heptanol level and exo-brevicomin presence, suggest an important interaction because of a positive effect between the higher level of heptanol and presence of exo-brevicomin. On the square root scale (employed for analysis), the interaction was not as obvious but was significant (\( t = 2.15, df = 20, P = 0.044 \)). Additional experiments are necessary to confirm the validity and magnitude of this interaction.

Heptanol level (averaged over presence/absence of exo-brevicomin) did not affect catch of \( T. \text{chlorodia} \) (high, \( n = 15 \), \( \bar{X} = 15.5 \pm 6.9 \) versus low, \( n = 10 \), \( \bar{X} = 4.6 \pm 1.2 \); \( df = 20 \), \( t = 1.29 \), \( P = 0.21 \)). For \( D. \text{valens} \), mean catch was significantly higher with the APT lure (\( \bar{X} = 96.8 \pm 3.6 \)) than with LRHHE (\( \bar{X} = 10.0 \pm 0.5 \) (Table 2). No other treatment differences were significant for \( T. \text{chlorodia} \) or \( D. \text{valens} \).

In experiment 2 (2007 late), a total of 1,021 \( D. \text{jeffreyi} \) (277 females, 739 males, 5 indeterminate), 7 \( D. \text{valens} \), 10 \( T. \text{chlorodia} \), and 7 Cleridae were caught (Table 3). Because of the low numbers of associates caught, we subjected only \( D. \text{jeffreyi} \) data to inferential statistics. The combination of verbenone and the two-component GLV mixture significantly reduced catch of \( D. \text{jeffreyi} \) by \( \approx 80\% \) compared with the attractant alone (\( t = 4.31, df = 22 \), \( P = 0.0003 \)). Ratio of females to males was unaffected by treatments (\( t = 1.76, df = 22, P = 0.25 \)).

In experiment 3 (2008), 4,506 \( D. \text{jeffreyi} \) (2,026 females; 2,469 males; 11 indeterminate), 127 \( D. \text{valens} \), 113 \( T. \text{chlorodia} \), and 22 Cleridae were caught (Table 4). \( D. \text{neuropterus} \) \( D. \text{jeffreyi} \) was significantly affected by

**Table 3.** Experiment 2. Mean catch of \( D. \text{jeffreyi} \) by semiochemical treatment at Luther Pass, CA from 19 July to 16 Aug. 2007 (\( X \pm 1 \text{SEM} \)). Associates were not evaluated due to their low numbers caught. Means within a column followed by different letters are significantly different by Tukey’s HSD (\( P < 0.05 \)). Columns without letters contain no significantly different means.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>D. jeffreyi</th>
<th>% females</th>
</tr>
</thead>
<tbody>
<tr>
<td>lure A*</td>
<td>70.7 ± 14.9a</td>
<td>27.7 ± 3.6</td>
</tr>
<tr>
<td>A + verbenone + GLVb</td>
<td>14.4 ± 3.1b</td>
<td>35.5 ± 5.5</td>
</tr>
</tbody>
</table>

* 95.5 n-heptane-1-heptanol released from 290-ml Nalgene bottle.

b Verbenone and GLV blend were released from separate pouches (BeetleBlock, Synergy Semiochemicals, Corp.).
treatment, with Lure A catching the greatest number; however, the only significant differences were between treatments with and without frontalin (Table 4). *Dendroctonus valens* was the only associate for which treatment effect was significant ($F_{4, 20} = 13.7, P < 0.0001$); each treatment with verbenone caught significantly fewer individuals than did those without (Lure A and Lure B; Table 4). Sex ratio of *D. jeffreyi* was not affected by semiochemical treatments in experiment 3 ($F_{3, 16} = 1.61, P = 0.23$ [treatment with frontalin excluded because of the low number caught]; Table 4).

In experiment 4 (2009), total catch of *D. jeffreyi* was 1,092 (491 females, 600 males, and 1 indeterminate), with 143 *D. valens*, 53 *T. chlorodia*, and 28 Cleridae also being trapped (Table 5). Semiochemical treatment was highly significant for explaining the number of *D. jeffreyi* caught ($F_{3, 16} = 32.2, P < 0.0001$). All treatments with frontalin differed significantly from Lure A; however, there were no differences among the disruptant treatments (Table 5). For *D. valens*, only the disruptant that included verbenone and GLV resulted in a noticeably lower mean catch; the only treatment effect was significant ($F_{4, 20} = 5.61, P = 0.0001$), which indicated that the disruptants, all of which contained frontalin, reduced the percentage of *D. jeffreyi* caught that was female.

Within each year of the study, the percentage of catch that was female using the blended 95:5 lure (APT or Lure A) decreased from start to end of the experimental period. On average, our first collection in a year was 59% female, whereas our last was 34% (Fig. 2). In 2007, our standard attractant treatments in experiments 1 and 2 were nearly identical (Table 1), yet the former (15 June to 18 July) caught 42% females and the latter (19 July to 16 August) 27.7%. Examination of the sex ratio at each collection period showed that the percentage of *D. jeffreyi* caught that was female declined throughout experiment 1 and leveled off for experiment 2 through about day of year 222 (10 August in nonleap years; Fig. 2). The linear decrease in the percentage female *D. jeffreyi* caught in the blended attractant during experiment 1 was significantly related to date ($n = 25$; r-square = 0.33; $P < 0.001$), dropping from a high of 55% on 20 June (second collection) to a low of 23% on 18 July (final collection). The average percentage female caught by Lure A in experiment 2 was 27.7 ± 3.6%; collection...
period had no linear effect on this value (n = 32; r-square = 0.003; P = 0.76). Lure A in experiment 3 (2008) averaged 51.0 ± 4.4% female; the female percentage decreased significantly with collection date (linear effect, n = 30, r-square = 0.33; P < 0.001) from 62.9 ± 5.3% on 2 July to 35.4 ± 7.7% on 6 August. In experiment 4, Lure A averaged 46.0 ± 2.1% female and again varied by collection period (linear effect, n = 41, r-square = 0.25, P < 0.001), ranging from 60.2 ± 3.3% on 6 July to 35.3 ± 10.9% on 3 August 2009. Combining across experiments provides a pattern of declining females until day of year 210–220 (29 July to 8 August in nonleap years), after which the percentage of females caught stabilized or even increased (Fig. 2). Overall, for the attractant treatments, the percentage of catch that was female dropped 4–8% per week from initiation of our trapping through day of year 220.

We measured semiochemical release rates in the field whenever possible, preferring these data over those generated in the laboratory. In experiment 1, the blended lure, APT, gave the highest release rate of n-heptane (estimated to be ~0.96 g/d at 26.2°C). However, all three high release heptanol treatments (APT, HRHH, and HRHHE) were estimated to have had similar release rates of 1-heptanol (Table 1). In experiment 1, the average measured loss of mass for field deployed n-heptane and 1-heptanol components in the HRHH and HRHHE lures was 550 mg/d after 17 d (at which time they were refilled) for n-heptane and 80 mg /d for 27 d for 1-heptanol. We estimate that the low heptanol treatments (LRHHE and LRHH) released ~6 mg/d over the same 27-d period in experiment 1. In experiment 2, the attractant blend (Lure A; 95.5 vol vol, n-heptane: 1-heptanol) was released from 250-ml bottles that began with 100 ml of mixture. Mean lost volume at the end of deployment was 42.7 ml, providing an average release rate of ~1.5 ml (~1 g) per day for the 29-d experiment (Tables 2–5). Release of verbenone, GLV, and frontalin was not measured at our study site, however, companion field trials were conducted in Missoula, MT and Pineville, LA during 2007 to provide field-based semiochemical release information (Table 1). Rates of ~66 mg/d for verbenone, 97 mg/d for GLV, and 6.8 mg/d for frontalin (600-mg load) were determined in Missoula in full sun at temperatures similar to those experienced at Luther Pass in the trapping portion of this study (Missoula mean temperature = 20°C; www.fs.fed.us/foresthealth/technology/elutionrate/lure.htm; Table 1).

**Discussion**

Commercially available racemic frontalin lures were potent disruptants of the *D. jeffrei* host selection process at the host or trap finding step. In experiments with frontalin over 2 yr, released at an estimated 6.8 mg/d from 600-mg lures and with or without verbenone and GLV, we caught 0.3 and 3.4% of the attractant-alone treatment. In addition, females were affected equally or to a greater degree compared with males. This level of disruption is rare with bark beetles, especially with the pioneering sex and a single disruptant (but see below for effects of MCH on *D. pseudotsugae*), and it suggests that successful protection of *P. jeffrei* may be achievable with a disruptant semiochemical.

Synthetic n-heptane and 1-heptanol lures were effective for capturing *D. jeffrei* in areas with elevated levels of host mortality. In each experiment, traps baited with the most effective attractant, a blend consisting of 95% heptane and 5% heptanol (vol:vol), caught at minimum 845 *D. jeffrei* (experiment 2) and up to 2,227 (experiment 3). Previous studies with *D. jeffrei* report best-lure catches of 56 in 6 wk (two traps per treatment; Renwick and Pitman 1979) or a range of two to 50 per day (duration and trap numbers unreported; Paine et al. 1999). In our study, the greatest numbers of *D. jeffrei* were caught in traps that released the most semiochemical (blended lure); however, differences were not significant among the three treatments that released high levels of heptanol in 2007 (estimated at 51–60 mg/d). The high heptanol treatments did, however, catch more *D. jeffrei* than the low heptanol treatments, supporting the finding of Paine et al. (1999) that increasing the release of heptanol may increase catch. In 2008, however, our blend of 75:25 heptane:heptanol (Lure B) did not catch more *D. jeffrei* (in fact 57% fewer) than the 95:5 blend (Lure A), suggesting that the effects of heptanol release are complicated by other factors.
Semiochemical treatments with exo-brevicomin did not significantly affect total catch of *D. jeffreyi* or the catch of females, but the number of males caught was reduced. The laboratory release rate of exo-brevicomin from the bubble caps used in this experiment was 125 μg at 20°C (Synergy Semiochemicals, Corp.; Table 1). Paine et al. (1999) found that exo-brevicomin released at 0.1% increased catch of females, but we do not know their quantitative release rate. Regardless, in our study, attractant blends were equally effective for attracting *D. jeffreyi* with or without exo-brevicomin.

Catch of *D. jeffreyi* insect associates was largely unaffected by our semiochemical treatments (Tables 2–5). Exceptions were attraction of *T. chlorodia* to exo-brevicomin and disruption of *D. valens* by verbenone and verbenone + GLV. Both of these effects were expected (Bedard et al. 1969, Gillette et al. 2001, Fettig et al. 2005, Zhang et al. 2007), but it is of interest to note that the disruption of *D. valens* was significant despite the differences between our *D. jeffreyi* attractant and the typical host monoterpenes used to attract *D. valens* (Zhang et al. 2007). For *T. chlorodia*, interaction plots suggest that the combination of heptanol release rate and exo-brevicomin presence were involved in attraction. This result was marginally significant and needs additional evaluation before their combined role will be clearer. The attraction of *T. chlorodia* to exo-brevicomin suggests that this compound should be deployed only after considering potential effects on this species.

In North America, the most successful example of tree protection with a semiochemical against a *Dendroctonus* bark beetle is the intraspecific pheromone MCH (3-methylcylohex-2-en-1-one) with *D. pseudotsugae* (Ross et al. 2002, 2006). Although rigorous multiyear or multilocation field studies during outbreaks are needed to prove treatment efficacy (Shea et al. 1984, Progar 2005), there are similarities between the frontal in-*D. jeffreyi* system and that of MCH-*D. pseudotsugae* that provide optimism. In trapping bioassays that preceded the development of specific methods for tree protection, MCH reduced trap catch of both sexes of *D. pseudotsugae* to 0 when deployed at 10 or 100% concentrations (estimated release rates of 5 and 50 mg/d, respectively; Rudinsky 1973). Our results with *D. jeffreyi* are similar, showing near complete disruption (both sexes) at the 6.8 mg/d level of frontal in. In *D. pseudotsugae*, MCH is produced by females who increase production in response to male stridulation (Rudinsky et al. 1973). At low concentrations MCH increases attraction; only at higher concentrations does it act as a disruptant of host selection. Thus, although produced by females, the disruptive functioning of MCH depends also on the presence of males. Frontalin is produced by male *D. jeffreyi* (Paine et al. 1999, Hall et al. 2002), as it is in *D. ponderosae* (Ryker and Libbey 1982, Pureswaran et al. 2000), and its disruptive effects may relate to attack cessation or shifting to another tree (Renwick and Vité 1970).

Frontalin is an attractant for several species of *Dendroctonus*, making nontarget effects (e.g., attracting other beetles to target or nearby trees) a consideration in its deployment. We are not aware that *P. ponderosa* is a host for other aggressive, tree-killing bark beetles, relegating nontarget impacts primarily to other hosts such as *P. ponderosa* Doug. ex Laws, *P. monticola* Doug. ex D. Don, *P. lambertiana* Doug., or *Pseudotsuga menziesii* (Mirbel) Franco. Frontalin is a component of semiochemical attractants for both *D. brevicomis* and *D. pseudotsugae* (Vité and Pitman 1969, Pitman and Vité 1970), but in both cases it is only a portion of a more complex semiochemical bouquet. Frontalin is not an attractant, and may be a disruptant, for *D. ponderosae* (Ryker and Libbey 1982, Pureswaran et al. 2000), so deployment should not promote undesirable attacks by this species. Field-testing under mixed stand conditions is necessary before the importance of these potential interactions will be understood. In this study we did not catch either *D. brevicomis* or *D. pseudotsugae* in our traps despite the elution of frontalin; we do not know if they were present in the area or not. We did, however, catch *D. valens*, a species for which the role of frontalin in its chemical ecology is uncertain or variable. Furniss and Schmitz (1971) working in Idaho characterize frontal in as a low level attractant, whereas Zhang et al. (2009) report significant disruptant effects in China. Regardless, frontal in did not impact our catch of *D. valens* in the presence of *D. jeffreyi* attractant (Tables 4–5).

This study shows that semiochemicals can impact behavior of *D. jeffreyi* in environments with ongoing tree mortality. The attractant blend of 95% n-heptane and 5% 1-heptanol consistently caught beetles over the 3 yr of our experiments, suggesting that it is a reasonable lure for monitoring this species. However, the seasonality of female catch in traps that we observed with this lure should be considered, especially when measuring possible control measures. Females are the pioneering sex and must be affected if preventing attacks is a goal: they responded to our traps in greater proportions early in the season. We do not know if the observed sex ratios in our traps were accurate indicators of the flying beetle populations or if there was a lure response interaction between date and sex. With *D. ponderosae*, Rasmussen (1974) working in the field, found that sex ratio was biased toward females by ~6% at the beginning of the emergence period, increasing to ~11% after 1 wk and falling to 2% after 18 d. Amman and Cole (1983) found a similar pattern in the laboratory. In both studies, sex ratio of emerging *D. ponderosae* favored females with the relative proportions changing during the emergence period but not dipping below ~1:1. More research is necessary with *D. jeffreyi* before interactions among time of emergence, response to lures, and sex are understood.

Frontalin was a strong disruptant for *D. jeffreyi* in this study when deployed alone, or in combination with verbenone and the two-component GLV blend of cis-3-Hexenol and 1-Hexanol (trap catches of nearly 0). A potential advantage of deploying the three-component blend is the possibility of also disrupting *D. valens*, a pest that is usually of minor importance in the
range of Jeffrey pine. However, disadvantages to operational use include the difficulties associated with registering multiple components with the USEPA (Gillette et al. 2006). Future studies will measure protection of high-value individual or groups of P. jeffreyi; however, until those studies are completed, frontal (with or without verbenone and GLV) may be the best option for those areas where D. jeffreyi is threatening individual trees or groups, but synthetic insecticides cannot be used.

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